

STUDIES ON SOME ANOMALOUS DICOTYLEDONOUS PLANTS.

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(WITH PLATES VII AND VIII)

HISTORICAL.

SEVERAL genera commonly referred to the dicotyledons differ from the typical ones both in the structure of the flower and in the form and arrangement of the vascular bundles. Among these anomalous dicotyledons are certain *Nymphaeaceae*, *Ranunculaceae*, and *Berberidaceae*, which have been referred to dicotyledons chiefly on account of the structure of the mature embryo.

The striking resemblance of the *Nymphaeaceae* to the *Alismales*, which are typical monocotyledons, has led to the study of the development of the embryo in certain genera of the former in order to determine the origin of the structures found in the mature embryo. The first work of this kind was done by Lyon (1901), who investigated the development of the embryo of *Nelumbo*. He found that the two large fleshy bodies of the mature seed seemed to be lobes of a single cotyledon which has its origin as a crescentic ridge of tissue, partly surrounding the plumule and later bifurcating to form what appears as two cotyledons.

Cook (1902) published the results of a study of the embryo sac and embryo of *Nymphaea advena* and *Castalia odorata*. He describes the embryo as monocotyledonous and agrees with Lyon that the *Nymphaeaceae* should be regarded as monocotyledonous.

In a paper which appeared a short time after that of Lyon, Campbell (1902) calls attention to the affinities of certain anomalous dicotyledons. It is pointed out that the embryo of the mature seed and the seedlings of certain *Ranunculaceae* and *Berberidaceae*, as observed by Lubbock and Holm, show the petioles of the cotyledons grown together where the edges are

in contact, thus forming a tube which extends down to the plumule and in some cases is quite long. In the light of Lyon's work on *Nelumbo*, the tube surrounding the plumule suggested to Campbell the possibility of a like origin of the cotyledons in these forms and a lateral origin for the plumule as in monocotyledons.

In the light of these facts, and because of our limited knowledge of the embryology of the so-called anomalous dicotyledons, it seemed desirable to study the embryology of several of these forms.

As the *Berberidaceae* include peculiar and varied plants, many of which differ widely from the typical *Berberis*, it was decided to begin work on certain representatives of that family, in which it seems that little of importance has been done on the development of the embryo. Holm (1899) has traced the development of *Podophyllum peltatum* from the seedling to the flowering plant, and both Dickson and Lubbock have observed the seedlings of *Podophyllum Emodi*, the Asiatic form. All agree in the description of a long cotyledonar tube with a very small plumule at the bottom, and that the cotyledons are peltate in form, the two together resembling the single leaf of the second year. Holm makes the statement that the cotyledons really represent a single leaf of this plant.

COLLECTION AND METHODS.

Material for all stages in the development of the embryo of *Podophyllum peltatum* and *Jeffersonia diphylla*, together with ripe seeds for germination, were collected during the spring and summer of 1902. The stages of *Caulophyllum thalictroides* important for this study were also secured. The material was fixed in 1 per cent. chrom-acetic acid, washed and brought into 70 per cent. alcohol, where it remained until ready for use. In the older seeds the outer seed coats were cut away in order to allow the fixing fluid to penetrate the tissue of the embryo. The sections were stained with safranin, Bismarck brown, gentian, and orange G. In this manner all morphological and cytological details are clearly brought out.

PODOPHYLLUM PELTATUM.

The mature embryo sac does not differ in any important particulars from that of typical angiosperms. The fecundated egg cell increases appreciably in size, becoming slightly elongated. The first division wall is transverse, and the two cells thus formed elongate rapidly, especially the one which is to form the suspensor. At this stage (*fig. 1*) one synergid is still to be seen near the embryo, but it does not persist for any length of time, as it was not observed in older stages. At this time several endosperm nuclei are seen scattered about within the embryo sac. The antipodal cells are distinct, but they soon begin to disintegrate and are not found in the stage represented by *fig. 5*.

The eight-celled embryo (*fig. 2*) is club-shaped, and the suspensor is rather long, consisting of two or three cells. The body of the embryo soon becomes broadened (*fig. 3*), and at the stage shown in *fig. 4* it is almost spherical. Growth now takes place most rapidly in the longitudinal direction, and at the same time the suspensor becomes somewhat thickened, its cells dividing longitudinally (*figs. 5, 6*). The embryo may now be well described as pear-shaped, although there is some variation in form at this stage. In some cases one side seems to grow more rapidly than the other, and an unsymmetrical form results (*fig. 6*). From the study of a large number of preparations, both longitudinal and cross-sections, it is highly probable that *fig. 5* represents the typical shape of the embryo shortly before the beginning of the development of the cotyledonar primordium. At this time the endosperm fills the entire embryo sac, except a small cavity around the embryo, which is partly filled by material from broken-down endosperm cells.

The origin and development of the cotyledons is peculiar. In typical dicotyledons the cotyledons are supposed to have their origin as two entirely separate and opposite ridges of tissue, standing at the same level, at the broadened distal end of the embryo, so that if cross-sections be made through the base of the cotyledons they would be separate at both sides in the first section in which they appear. Although this mode of origin has been assumed by certain writers, it seems that comparatively

little has been done in the investigation of the origin of the cotyledons in the typical dicotyledons, especially by the use of series of cross-sections which are often indispensable in getting the correct orientation of parts. It may be that in many so-called typical dicotyledons there is much variation from what has been described as the accepted origin of the cotyledons. This can be determined only by a thorough and detailed study of a large number of forms.

In *Podophyllum* the primordium of the cotyledons appears first as a rather broad outgrowth from the margin of the truncated distal end of the embryo, forming an almost complete ring. This is made clear by a series of cross-sections such as *fig. 6*. In this figure *a* and *b* are sections through the broad part of the embryo just beneath the primordium of the cotyledons; *c*, through the plumule and the base of the ridge; *d-l* include the remainder of the cotyledonar primordium. From this figure it is apparent that the cotyledons arise as a single primordium which consists of a circular ridge broken at one side by a notch or indentation. As soon as the ridge has attained a little height, a notch is formed on the opposite side, and we have now two divisions or lobes which will develop into what are considered as two cotyledons. The figure shows that one lobe is slightly longer than the other; but this is not of importance, because in other preparations the two were found frequently to be of equal size.

A later stage is shown in *fig. 7*. The bottom of the first notch together with the cotyledonar primordium has undergone intercalary growth, so that a very short tube has been formed. This series shows that both the ridge and the lobes have increased in height, but the lobes have grown the more rapidly, because their height is now about equal to that of the ridge (compare *fig. 6*), and are of equal size. The open ridge is more prominent at this time than in younger or older stages, the notch first formed being about twice as deep as the other. The edges of the ridge are in contact below, but above they are separated. The growth of the tissue at the bottom of the first notch goes on more rapidly than that at the bottom of the second notch, so that the two tend to become equalized. This greatly increases the length of the

cotyledonar tube. *Fig. 8* represents a series of sections from an embryo a little older than the one represented by *fig. 7*. Only alternate sections were drawn. This series makes apparent the great increase in the length of the tube and the almost equal depth of the bifurcations. It seems that the bifurcations do not become of exactly the same depth at any time, as an examination of a number of series from mature embryos did not show them more nearly equal than they are in the stage represented by this figure.

It is very necessary to use series of cross-sections in tracing this development. Median longitudinal sections at right angles to the plane of bifurcation of the cotyledonar ridge present the appearance shown in *figs. 10, 11, 12*, and cannot be distinguished from similar sections of a typical dicotyledon. The ring of tissue and its two divisions increase greatly in length. There does not seem to be a localization of growth in the lobes here, as described for *Nelumbo*, but the ring itself grows at about the same rate as its two lobes. A narrow opening extends down to the growing point of the stem. The opening is conical just above the plumule, but for most of its length the walls of the tube are in contact.

The hypocotyl is short, and the plumule is but little developed in the mature embryo. The growing point of the stem shows simply a slight protuberance into the conical opening at the base of the cotyledonar tube. The cells of this mound of tissue are slightly different in size and staining properties from those around them (*fig. 13*).

The conducting tissue begins to develop as a central strand of narrow elongated cells, which extends almost to the plumule, where it divides, one division going to each of the cotyledons. The form of the mature embryo is shown by *fig. 14*, which is a diagram to represent a median longitudinal section of a mature embryo. The figure was constructed from micrometer measurements of a series of cross-sections of known thickness. This was found more satisfactory than making a drawing from a longitudinal section, because in the latter it is impossible to distinguish the place where the cotyledonar tube leaves off and the opening between the two lobes begins.

JEFFERSONIA DIPHYLLA.

The development of the embryo sac of *Jeffersonia* has been described by Andrews (1895). The mature embryo sac differs from the typical form only in the large size of the antipodal cells. The fecundated egg cell elongates and is divided into two cells by a transverse wall (*fig. 15*). The embryo soon elongates, the distal end becomes somewhat broadened, and a definite suspensor consisting of two or three cells is formed. The cells at the distal end are divided by longitudinal walls, but the suspensor remains as a single row of cells (*figs. 16, 17, 18*). In *fig. 18* a synergid is shown which has increased greatly in size. This is an unusual occurrence, as the synergids usually disappear early. By the time the embryo has reached the eight-celled stage a large number of endosperm nuclei can be seen scattered about in the cytoplasm of the embryo sac. The antipodal cells disappear very early.

The suspensor increases in length by the growth and division of the cells composing it. The cells divide by transverse walls until a row of four or five cells is formed. At first the body of the embryo seems to increase in size by the division of its cells in three planes, so that it becomes rounded (*fig. 19*). A little later the body elongates, and the symmetrical form shown in *fig. 20* is produced. Growth now takes place most rapidly in a lateral direction, the distal end becoming broad and truncate. *Fig. 21* represents a stage just before the beginning of the development of the cotyledons. The development of the embryo to this point corresponds very closely with that described for *Podophyllum*. The cavity surrounding the embryo is small, and the endosperm cells near it do not become so nearly disorganized as in *Podophyllum* or *Caulophyllum*.

Examination of longitudinal sections, or of series of cross-sections of embryos in which the cotyledons are somewhat developed, would lead one to the conclusion that *Jeffersonia* is a typical dicotyledon (*figs. 22, 23*). There is a slight peculiarity in the origin of the cotyledons, however, which is shown by a series of cross-sections at the stage when the cotyledonar primordium first appears. *Fig. 24* shows that the cotyledons really

have their origin as a very broad crescentic ridge of tissue. After the ridge has attained a little height it bifurcates, so that two lobes are formed.

In older stages the two lobes are of equal size and the notches are of almost equal depth. *Fig. 23* represents the condition in the mature embryo. Section *a* passes through the body of the embryo just below the growing point; *b* passes through the plumule; the remaining sections pass through the cotyledons. It will be observed that no cotyledonar tube is formed and that the edges of the cotyledons are not close together.

The mature embryo is small, both hypocotyl and cotyledons being short. The plumule has about the same development here as in the mature embryo of *Podophyllum*, being simply a small mound of tissue between the two cotyledons. The conducting tissue is beginning to develop as a central strand of narrow elongated cells, which divides into two branches, one going to each of the two cotyledons. *Fig. 25* shows the shape of the mature embryo in longitudinal section.

CAULOPHYLLUM THALICTROIDES.

Material for the study of the younger stages in the development of the embryo of *Caulophyllum* was not secured, but it is hoped that the investigation of the development of the embryo-sac and young embryo may soon be carried out.

The youngest stages examined show the embryo at about the time when the cotyledons begin to develop. The embryo at this time shows a long, slender suspensor and a rather broad, short body (*fig. 26*). From this longitudinal section the cotyledons seem to arise as two opposite ridges of about equal size, but it is impossible to say that this is the case, as cross-section series of this stage were not secured and series from embryos a little older do not support this view. A cross-section series from an embryo corresponding in size to the one represented by *fig. 27* is shown in *fig. 28*. From this figure it is apparent that the cotyledons have a common base consisting of a low ridge of tissue which bifurcates to form two very symmetrical cotyledons. Older stages show that the bifurcations soon become of almost equal

depth (*fig. 29*). The edges of the cotyledons are very close together, so that the plumule is almost inclosed in a tube. The opening just above the plumule is conical as in *Podophyllum*. The plumule is slightly more developed here than in *Podophyllum* or *Jeffersonia*, but consists simply of a mound of tissue. The hypocotyl is longer in relation to the size of the entire embryo than in either of these forms.

SUMMARY.

1. In each of the three plants studied the embryo is small, surrounded by a large amount of endosperm richly stored with starch.

2. In each case the cotyledonar primordium is a broad, ridge-like structure open at one side. This ridge later bifurcates opposite the opening to form two lobes, the cotyledons. The two notches soon become of almost equal depth, so that older stages do not give a correct impression of the origin of the cotyledons.

3. In *Podophyllum* a long cotyledonar tube is formed, and in *Caulophyllum* the edges of the cotyledons are close together. The plumule of *Jeffersonia* is not inclosed in either way.

4. The plumule is not well developed in any of these embryos, but has a central origin in each.

THEORETICAL CONSIDERATIONS.

There are among the angiosperms certain plants which show peculiarities either in their vegetative structure, their embryology, or in both. Some of these plants have flowers of very simple form, and certain botanists believe that in them we have primitive characters, and that the comparative study of a large number of these forms may reveal much concerning the origin of the structures found in more highly developed plants. Others hold that the simple forms represent merely a derived condition, and that their peculiarities are not of the highest value in determining relations, but have been acquired as an adaptation to the conditions under which the plant has developed. All agree that it is important to investigate the development of these plants, because it is only by the accumulation of a large number of facts that we have a basis for definite and reliable conclusions.

There are two points of great interest in the study of these low plant forms, when they are considered as representing primitive characters: first, the possibility of determining something concerning the origin of the angiosperms; and, second, the origin and order of derivation of the monocotyledons and dicotyledons.

The gap existing between the seed-bearing plants and the pteridophytes of the present time is wide, the nearest approach to the seed habit being found in the few heterosporous forms. It seems probable that the seed-bearing plants had their origin from heterosporous pteridophytes of past ages, and that the Gymnosperms and Angiosperms arose independently from different stocks. On the other hand, from certain similarities it seems probable that the monocotyledons and dicotyledons are derived from a common stock. If these two divisions had a common origin, the question arises as to which is the more primitive and how the other has been derived from it. There are two theories concerning this which are diametrically opposed.

The theory advanced by Lyon (1901) is that the single cotyledon is homologous with the foot of the pteridophytes, and that the dicotyledons are derived from the monocotyledons by the bifurcation of the single cotyledon as in *Nelumbo*. However true this theory may prove to be, it seems necessary that it should be substantiated by a larger collection of facts before it can be of great value. If the study of a large number of anomalous forms should show the ridge of tissue at the base of the cotyledons of varying height, so that a connected series could be built, it would in some measure support this theory, although even then the conditions under which the plants develop would have to be considered, as the peculiarities might represent simply derived conditions.

Opposed to this theory is that of Miss Sargent (1903) set forth in her paper on the origin of the monocotyledons. This observer argues that the monocotyledons are derived from the dicotyledons, the single cotyledon of the former having arisen by the union of the two cotyledons of the latter, and that therefore the dicotyledonous plants are the more primitive. This

theory is based on the study of the seedlings of a large number of plants, many of which show the cotyledons grown together at one or both sides.

To determine which of these theories is the more probable will require the study of the embryology and development of the seedlings of many plants in the families which show these peculiarities. Something of value may be learned by growing the seedlings under different conditions and observing results.

The interpretation of such a structure as that found in *Podophyllum*, or that of *Nelumbo* as one cotyledon or as two, must be unsatisfactory until a greater number of anomalous forms has been investigated. If the cotyledons of some of the plants which have the other characters of the dicotyledons are found to have their origin as a single ridge, it will support the view that here also we have two cotyledons and not lobes of one. The early division of the ridge in *Podophyllum*, as well as in *Jeffersonia* and *Caulophyllum*, leads to the conclusion that here we have two cotyledons. The fact that a cotyledonar tube is formed in *Podophyllum* is probably best explained as a derived condition brought about by the geophilous habit of the plant. The hypocotyl is short in comparison with the length of the entire embryo. The plumule is small and develops but little during the first year. The cotyledonar structure is long, so that, while the plumule remains underground in a protected position, the cotyledons are pushed up into the air and sunlight and carry on the work of assimilation. The great length of the cotyledons makes necessary some means of strengthening the part which must support the weight, and this is done by the formation of the tube. The fact that cotyledonar tubes have been found in families far removed from each other, and usually in embryos having a short hypocotyl, supports this view.

In *Caulophyllum* the ridge does not attain much height, and the later stages show that the notches are of almost equal depth and that the cotyledons are very symmetrical. The hypocotyl is long and the cotyledons short in comparison with *Podophyllum*. Possibly this explains why no tube is formed.

It is highly desirable to trace the development of the seed-

lings of *Jeffersonia* and *Caulophyllum* for comparison with the conditions found in *Podophyllum*. Up to this time (March 20) seeds of *Podophyllum* and *Jeffersonia* collected last year have refused to germinate. It seems that they require a long resting period and will not germinate until the spring following their maturity. It is hoped that this year the germination of the seeds and the development of the seedlings may be observed.

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EXPLANATION OF PLATES VII AND VIII.

PLATE VII.

FIGS. 1-14. *Podophyllum peltatum*.

FIG. 1. Section of two-celled embryo with large synergid. $\times 180$.

FIG. 2. Eight-celled embryo. $\times 180$.

FIGS. 3-4. Embryos with broad or rounded body and long suspensor $\times 180$.

FIG. 5. Embryo shortly before the beginning of the development of the cotyledonar primordium. $\times 180$.

FIG. 6. A series of cross-sections from an embryo showing the cotyledonar primordium; the sections of all the series shown in the figures are 15μ thick. $\times 25$.

FIG. 7. A series of sections from an older embryo, showing the beginning of the cotyledonar tube, and the bifurcation of the ridge to form two cotyledons. $\times 25$.

FIG. 8. A series of sections from an embryo in which the cotyledonar tube is somewhat developed; the bifurcations are of almost equal depth; alternate sections were drawn. $\times 25$.

FIG. 9. Embryo of irregular shape. $\times 180$.

FIG. 10. Longitudinal median section of an embryo at right angles to the plane of bifurcation of the cotyledonar ridge. $\times 100$.

FIGS. 11-12. Similar to *fig. 10*, but later stages. $\times 100$.

FIG. 13. Longitudinal section through the plumule of a mature embryo. $\times 180$.

FIG. 14. Longitudinal section of a mature embryo of *Podophyllum*. $\times 25$.

PLATE VIII.

FIGS. 15-25. *Jeffersonia diphylla*.

FIG. 15. Two-celled embryo. $\times 180$.

FIG. 16. A later stage to show that the suspensor becomes long, before longitudinal walls are formed in the cells at the distal end. $\times 180$.

FIG. 17. Eight-celled embryo with large synergid near it. $\times 180$.

FIG. 18. Similar embryo with endosperm nuclei in cytoplasm of embryo sac. $\times 100$.

FIG. 19. Older embryo in which the body is somewhat rounded. $\times 180$.

FIG. 20. Embryo in which the body is more elongated than in *fig. 15*. $\times 180$.

FIG. 21. Form of embryo just before the beginning of the development of the cotyledons. $\times 180$.

FIG. 22. An embryo in which cotyledons are almost developed. $\times 100$.

FIG. 23. A series of sections from a mature embryo.

FIG. 24. A series of cross-sections of an embryo, showing that the cotyledons begin their development as a single broad ridge. $\times 25$.

FIG. 25. Longitudinal section of a mature embryo. $\times 25$.

FIGS. 26-30. *Caulophyllum thalictroides*.

FIG. 26. Embryo at beginning of development of cotyledons. $\times 180$.

FIG. 27. Older embryo. $\times 100$.

FIG. 28. A series of sections through the plumule and base of the cotyledons. $\times 25$.

FIG. 29. A series of sections from an older embryo; the first eight sections are consecutive, the others are alternate. $\times 25$.

FIG. 30. Longitudinal section of a mature embryo. $\times 25$.